1228-Pos Board B138
Computational Modeling of Cardiac Troponin Dynamics: Elucidating a Regulatory Mechanism for Calcium Activation of the Thin Filament
Edward P. Manning, Jil C. Tardiff, Steven D. Schwartz.
Calcium activation of the thin filament regulates contraction of skeletal and cardiac muscle via the protein complex troponin and its interaction with tropomyosin. The mechanism of this regulation remains mostly unknown. A complete dynamic, atomistic model of cardiac troponin is developed, constructed from various structures and models. The mechanism underlying cardiac troponin regulation of the thin filament is investigated using molecular dynamics simulations of the model in two states: calcium bound and not bound to site II of the N-lobe of cardiac troponin C. Significant changes in dynamics of various regions are observed throughout the cardiac troponin complex and in the overlapping tropomyosins. This leads us to believe that calcium-dependent alterations in dynamics propagate throughout the cardiac troponin complex and alter tropomyosin dynamics, resulting in calcium-dependent activation of the thin filament.

1229-Pos Board B139
Novel DNA Target Site Search Mechanism for Sequence-Specific DNA Binding Proteins
Markita P. Landry, Yann R. Chemla.
A significant subset of protein-DNA interactions occur between proteins that identify a specific target sequence amongst large sections of nonspecific DNA. The currently accepted model for this process states that sequence-specific DNA-binding proteins (SSDBPs) find their DNA targets by a ubiquitous process in which they bind loosely to DNA to scan DNA in 1-D, adopting a tightly bound state to the DNA only at the target sequence1. However, little consideration has been made for the vast array of properties exhibited by SSDBPs which could influence protein search behavior prior to finding the DNA target site. SSDBPs differ considerably in size, DNA target sequence length, function, and (in) dependence on cofactors. These properties affect protein activity at the target sites and may similarly affect protein search mechanisms.
We propose that SSDBP search mechanisms may not be ubiquitously defined for all SSDBPs. We study protelomerase TelK, a cofactor-independent single-turnover protein responsible for the formation of DNA hairpins in prokaryotic DNA, and its mechanism of DNA target site identification2. High resolution optical trap studies show an unpredicted observation of nonspecific DNA condensation by TelK. TIRFM studies show that TelK 1D scanning of nonspecific DNA occurs only at low concentrations where TelK is a monomer. 1D scanning ceases at higher TelK concentrations, as TelK forms aggregates of multiple TelK units. This suggests that TelK searches for its target site while loosely bound to DNA as a monomer and aggregates once it encounters another TelK unit on DNA, before reaching the target site. These results suggest a mechanism contrary to the currently accepted model for SSDBP targeting mechanisms, indicating that these processes may vary from protein to protein.
1. Halford et al. Nucl. Ac. Res 32, 3040 (2004).
2. Aihara et al. Mol. Cell 27, 901 (2007).

1230-Pos Board B140
Molecular Dynamics Simulation of a Spin-Labeled Membrane Protein
Alexandra M. Zudova, Bengt Svensson, Michel Espinoza-Fonseca, David D. Thomas.
We have developed a computational molecular dynamics technique to simulate the motion of the TOAC spin label bound to the cytosolic and transmembrane domains of monomeric phospholamban (PLB) in a lipid bilayer. In cardiac muscle, the integral membrane protein PLB binds to and inhibits the Sarco/Endoplasmic Reticulum Ca^{2+} ATPase (SERCA), decreasing calcium sequestration into the SR lumen. Previous electron paramagnetic resonance (EPR) experiments showed strong immobilization of the spin probe when placed near the C-terminus of PLB, indicating a stable and highly ordered transmembrane helix. It was shown that the cytosolic domain samples two distinct conformations, an ordered T state with moderately restricted motion and a dynamically disordered R state with nearly unrestricted isotropic motion. Phospholamban regulatory function requires an order-to-disorder transition in the cytosolic domain, with phosphorylation at Ser16 shifting the equilibrium toward the mobile R state and relieving SERCA inhibition. In the present study, monomeric PLB was simulated in a POPC lipid bilayer with the newly parameterized TOAC spin label replacing amino acids at positions 11 and 36. Unlike cysteine-reactive spin labels, TOAC directly reports the motion of the peptide backbone, making it possible to computationally analyze the coupled motion of the transmembrane helix. The simulation for each mutant is performed with and without phosphorylation of Ser16. We optimized molecular dynamics simulation conditions and the resulting trajectories are used to calculate order parameters, orientational distributions, rotational correlation times, and helical tilt angles for comparison with previous experimental results. The dynamics of the spin-labeled protein are compared with the wild type monomer to investigate the effect of the spin probe on the peptide backbone. This computational method informs our current experimental model of TOAC-protein dynamics and strengthens our molecular dynamics modeling technique for performing future spin probe experiments *in silico*.

1231-Pos Board B141
Rotational Power Spectra of Protein Backbone
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The hierarchal levels of internal movements of proteins may determine their functional processes; however the functional role of torsion vibrations of protein backbone is still ambiguous. We study the characteristic features of protein backbone vibrations and their relations with the protein conformational states. We used molecular dynamics simulations to determine the backbone rotational angles of each amino acid with femtosecond resolution in different conformational states. The time series of the angles were Fourier transformed and filtered to produce the power spectra of the rotations. For each amino acid, characteristic rotational spectra consisting of specific peaks in the 5-60 THz (150-1800 cm-1) region were found beside the lower frequency normal torsional peaks of free amino acids. The positions of the spectral peaks were stable on the nanosecond timescale. Interestingly, amino acids of the proteins could be classified into 10 distinct groups based on the specific part (5-60 THz) of their spectra. Very similar classes can be found in different proteins. We observed that the spectra of certain functional amino acids were altered between different conformational states.

1232-Pos Board B142
Protein Structural Mode Separation with Modulated Orientation Sensitive Terahertz Spectroscopy
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The far infrared spectroscopy of molecular crystals reveals both intra-molecular and intermolecular vibrational modes. Such spectroscopic measurements may also be used for protein crystals to detect correlated structural motions necessary for function. However with the significant increase in complexity of protein structures compared to simple molecules such as sucrose, one finds increasing overlap in the internal modes. Here we demonstrate a new technique called MOSTS (Modulated Orientation Sensitive THz Spectroscopy). We achieve high sensitivity and mode separation by using single protein crystal and rapid modulation of the relative alignment of the terahertz polarization and the crystal axes by rotating the sample. By locking into the signal at the rotation frequency we determine the polarization sensitive signal as a function of phase and map out the optically active vibrational resonances for different orientations of the molecular crystal. The method is only sensitive to the anisotropic part of the spectroscopic response.
To illustrate the technique we compare two methods of detection. First, the signal modulated by generating antenna bias, the standard Terahertz time domain spectroscopy (THz TDS) method and the second, the MOSTS method where the signal is modulated by the sample orientation. We present measurements on a wire grid linear polarizer, a sucrose crystal and a hen egg white lysozyme crystal. This work is supported by NSF MRI^2 grant DBI2959989.

1233-Pos Board B143
Further Developement of Rotational Symmetry Boundary Condition: Simulation of Pentamer in Icosahedrally Symmetric Virus Capsid
Shigetaka Yoneda, Maiko Nanao.
The rotational symmetry boundary condition (RSBC) is an approach to accelerate simulations of rotationally symmetric assemblies of macromolecules. As structures of many virus capsids are icosahedrally symmetric, we have applied the RSBC to some virus capsids; the simulations have been restricted to one (black in the figure) of the 60 symmetric units of capsid by the RSBC. However, asymmetric deformation around the 5fold axis, including motion of ions, can not be calculated in the present RSBC program. Thus to include asymmetry at the 5fold axis, further development of RSBC was performed using a pentamer composed of 5 asymmetric units (black and gray in the figure) for computational cell. We present a set of computational tools needed for the pentamer-based RSBC simulation, including rotation matrices, vectors of rotation axes, partition planes of cells, a table of cell contacts, a group table needed for the protomer number equation, a cell-number table, and border regions to identify atoms near cell borders. Short computational analysis on a rhinovirus capsid is also presented with the newly-developed RSBC program.